P90

RETROVIRAL MEDIATED RNA INTERFERENCE TO KNOCKDOWN MATRIX METALLOPROTEINASE 13 IN HUMAN OSTEOARTHRITIC CHONDROCYTES

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Aim: To test the feasibility of retroviral mediated RNA interference to stably knockdown the expression of MMP-13 in human osteoarthritic (OA) chondrocytes in 3-D cultures.
Methods: Primary human chondrocytes were efficiently transduced with amphotyped pSUPER.retro moloney retroviruses harboring Hu MMP-13 specific short hairpin RNAs (OE7 and OE16). Chondrocytes, obtained from 7 patients undergoing knee arthroplasty were infected at their first passage. To investigate the functional efficacy of MMP-13 knockdown in a culture model mimicking differentiated chondrocytes in close contact with their extracellular matrix, puromycin resistant populations of stably transduced cells were seeded into micromass cultures. Micromasses were either left in basal conditions or stimulated with IL-1β, GROα, SDF-1 or BCA-1. Supernatants were assayed by ELISA to measure the concentrations of MMP-13 and of collagen II degradation products.
Results: Populations of primary chondrocytes from different patients, stably expressing the OE7 and OE16 shRNAs, showed greater than 80% MMP-13 knockdown by quantitative real time PCR in comparison to the same cells expressing a negative control luciferase shRNA (pSUPER.retro GL2). In keeping with these data, ELISA quantification of cell supernatants also revealed strong inhibition of stimulated MMP-13 release and also lower basal levels in the OE7 and OE16 compared to the GL2 control chondrocytes. In keeping with these observations, the release of collagen II degradation products was also specifically abrogated in the same cells exhibiting penetrant MMP-13 knockdown.
Conclusions: RNA interference technology coupled to efficient retroviral delivery is an effective tool to stably silence MMP-13 in OA chondrocytes, and thus reduce ECM degradation. Targeting either other unwanted matrix degrading enzymes or the signaling pathways, which determine their expression may be an effective means to limit cartilage destruction.

P92

REGIONAL GENE THERAPY FOR FULL-THICKNESS ARTICULAR CARTILAGE LESIONS USING NAKED DNA IN A COLLAGEN MATRIX

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Aim: This study aimed to establish a novel in vivo gene delivery system for repair of articular cartilage lesions.
Growth factors have been used to facilitate cartilage repair. However these factors are expensive, require large therapeutic doses, may be degraded, diffuse away from the desired location, and/or require activation of a latent form. Regional genetically engineered constructs may circumvent these problems. Gene therapy for arthritis has focused on viral vectors to introduce the desired gene. Concerns regarding these vectors include insertional mutagenesis, difficulty targeting specific tissues, and immunogenicity to viral particles. Nonviral gene therapy (naked DNA) is an alternative to viral-mediated gene transfer.
Methods: Human BMP-2 cDNA was cloned into pc.DNA3(+), creating the plasmid "pc.hBMP2". Lipopolysaccharide–free DNA was prepared. 1.Functional expression of BMP-2 was examined by transfecting pc.hBMP2 into 293T cells and testing conditioned medium for osteogenic differentiation capability, by measuring alkaline phosphatase (ALP) activity in W20-17 murine marrow cells. 2.Diffusion kinetics of plasmid from collagen sponges in synovial fluid under constant agitation was examined.
3.Osteochondral defects were created in the femoral trochlea of skeletally mature rabbits. In each of three rabbits, one defect received a collagen sponge with p.c.b-gal plasmid; the contralateral defect received a sponge with control plasmid, to show that naked DNA is taken up and expressed in this model. Histological sections prepared at 1 week were immunostained with antibody to b-gal. 4.Defects, created as above, were treated with one of four implants: collagen sponge only (N = 9);sponge containing control plasmid (N = 12); sponge containing p.c.hBMP-2 (N = 12); or sponge containing rhBMP-2 protein (N = 12). Animals were sacrificed at 12 weeks and histology prepared.
Results: ALP activity in W20-17 cells increased in response to conditioned medium. A continuous slow diffusion of plasmid DNA from the sponge was seen for 18 hr, at which time the sponge retained $>$ 98% of the plasmid DNA. Histology showed mesenchymal-appearing cells deep to the defect expressing b-gal at 1 week after implantation. Differentiated bone cells, chondrocytes, and synoviocytes were unstained. Lesions treated with pc.hBMP2 plasmid induced hyaline-like cartilage repair (score 18.8 $\pm$ 3.8) nearly equivalent in quality to that induced by collagen sponges with recombinant BMP-2 protein (score 20.3 $\pm$ 2.5).
Conclusion: The novel gene therapy approach of implanting endotoxin-free 'naked' cDNA containing the gene for BMP-2 in type I collagen sponges into cartilage defects successfully induced repair at 12 weeks. Our approach circumvents the risks of inflammation and immunogenic response associated with the use of viral vectors. Naked plasmid DNA as a vehicle for transferring therapeutic genes is a potentially important clinical means of facilitating articular cartilage regeneration and is both safe and cost-effective.

P93

ANGIOGENESIS IN HUMAN AND GUINEA PIG OSTEOARTHRITIS

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Aim: To compare osteochondral angiogenesis in spontaneous OA between man and Dunkin Hartley (DH, susceptible) and Strain 13 (S13, less susceptible) guinea pigs.
Methods: Central weight-bearing portions of human medial tibial plateaux (MTP) were obtained at total arthroplasty for OA (n = 45), or *post mortem* from people with no macroscopic evidence of OA (PM) (n = 14). Right knees were obtained from DH guinea pigs aged 3, 6, 9 and 12 months (n = 6 per age) and from S13 guinea pigs aged 3 (n = 4) and 12 months (n = 3). Vascular densities (No. vessels breaching osteochondral junction mm^{-1}) were